Human Cortisol ELISA Kit

Enzyme Immunoassay for the quantification of free Cortisol concentration in Human urine.

Catalog number: ARG82799

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Cortisol is a steroid hormone, in the glucocorticoid class of hormones. When used as a medication, it is known as hydrocortisone.

It is produced in many animals, mainly by the zona fasciculata of the adrenal cortex in the adrenal gland. It is produced in other tissues in lower quantities. It is released with a diurnal cycle and its release is increased in response to stress and low blood-glucose concentration. It functions to increase blood sugar through gluconeogenesis, to suppress the immune system, and to aid in the metabolism of fat, protein, and carbohydrates. It also decreases bone formation. [Provide by Wikipedia: Cartisol]

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. An antibody specific for Cortisol has been pre-coated onto a microtiter plate. Endogenous Cortisol of samples, Standards and Controls competes with a Cortisol-HRP Conjugate for binding to the immobilized antibody. After incubation and washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of AMH bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of AMH in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C in the dark. Open the bag of Coated Microplate only when it is at room temperature and close immediately after use. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated microplate	8 X 12 strips	4°C
Standards A (0 ng/mL)	4 mL (ready to use)	4°C
Standards B to E (1, 5, 30, 200 ng/mL)	I mL each (ready to use)	4°C
Control 1 & 2	I mL (ready to use)	4°C
Cortisol-HRP Conjugate	33 mL (ready to use)	4°C
10X Wash Buffer	50 mL	4°C
TMB substrate	15 mL (ready to use)	4°C (protect from light)
STOP solution	15 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Mixer or Ultra-Turrax
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (22-28°C).
- Open the bag of Coated Microplate only when it is at room temperature and close immediately after use.
- The clinical significance of the Cortisol determination can be invalidated if the patient was treated with corticosteroids or natural or synthetic steroids.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the

crystals are completely dissolved.

- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Urine: The total volume of urine excreted during 24 hours should be collected and mixed in a single container. Urine samples which are not to be assayed immediately should be stored at 2-8°C or at-20°C for longer periods (maximum 6 months). Avoid freeze-thaw cycles.

Note:

1. The kit has been designed to be used on untreated urine samples; acidification treatments of the urine that lead the pH to values below 5.0 could interfere with the assay and produce aberrant results.
2. Samples containing sodium azide should not be used in the assay.
3. Samples with concentration greater than 200 ng/mL have not to be diluted; such samples have to be reported as "> 200 ng/mL".

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 50 mL of 10X Wash Buffer into 450 mL of distilled water to a final volume of 600 mL) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-26°C) for at least 30 minutes before use. Standards and samples should be assayed in duplicates.

1. Add **10 µL** of **samples, Controls and Standards** to the **Antibody Coated microplate**.
2. Add **300 µL** of the **Cortisol-HRP Conjugate** to each well. Thoroughly mix for **10 seconds**. **It is important to have a complete mixing in this step.**
3. Incubate at **37°C** for **1 hour** on a microplate shaker.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with **1× Wash Buffer (350 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µL** of **TMB Substrate** to each well, including the blank wells. Incubate for **15 minutes** at **RT** in the dark.
6. Immediately Add **100 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
7. Read the OD with a microplate reader at **450 nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance **within 5 minutes** after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be reported as > 200 ng/mL.
6. To calculate the cortisol concentration in urine, calculate as above and correct for total volume of volume of urine collected in 24 hours:

   ng/mL x Vol (mL) urine 24 h / 1000 = µg Cortisol/24 h
7. The reference value of Cortisol in urine is 1.5-63 µg/24h.

QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of Cortisol is 0.47 ng/mL.

Cross Reactivity

Substance	Conc.	Interference
Albumin	5 mg/dL	No
Acetylsalicylic acid	3.62 mmol/L	No
Ibuprofen	2.42 mmol/L	No
Ascorbic acid	5 mg/L	No

Intra-assay and Inter-assay precision

The CV value of intra-assay was 6.6-8.1% and inter-assay precision was 9-12%